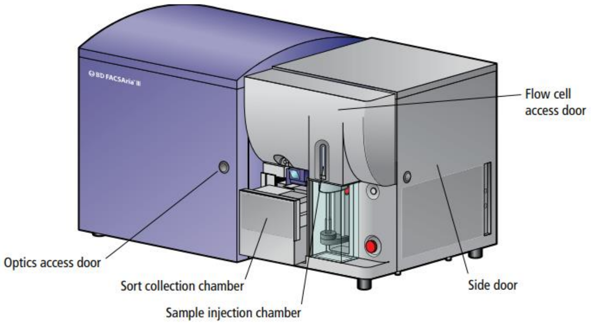
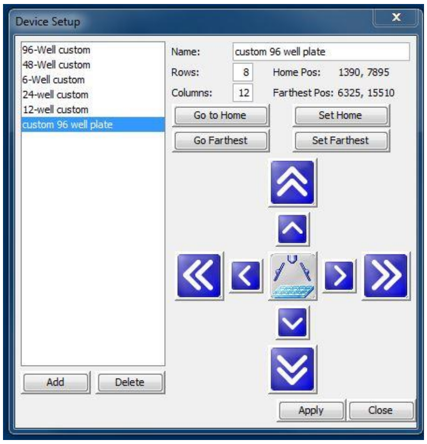
Memorial Sloan Kettering Cancer Center

FACSAria™ custom device SOP




Device Setup

Name: custom 96 well plate
Rows: 8
Columns: 12
Home Pos: 1390, 7895
Farthest Pos: 6325, 15510

Flow Cytometry
Core Facility

Ver. # 1.0
2020-06-26

Workflow Overview

Create a custom device in Diva by setting up the number of rows and columns and setting up the position of the home and farthest. Diva software calculates the increment between rows and columns to determine the sort locations.

Create a new experiment ▶ Set up custom plate ▶ Test sort with beads ▶ HRP method (optional) ▶ Start sort experiment

Purpose

Introduction on how to program ACDU stage for custom device (such as 96 well PCR plate) on FACSAria™.

1. Create a new experiment

1. Start up system and run QC on FACSAria™ with the 100 µm nozzle (3-peak bead check and drop charge delay verification).

   Note: Check ▶***FACSAria™ Startup, QC & Shutdown Procedure***.

2. Create a *New Experiment* in Diva and adjust all instrument settings specific to your needs.
3. Set up compensation and calculate compensation if needed.
4. Acquire and record sample. Create plots and gates based on your experiment strategy.

2. Set up custom plate

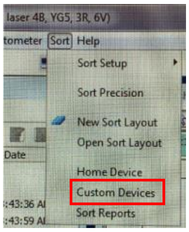
1. Install the splash shield below the aspirator drawer.

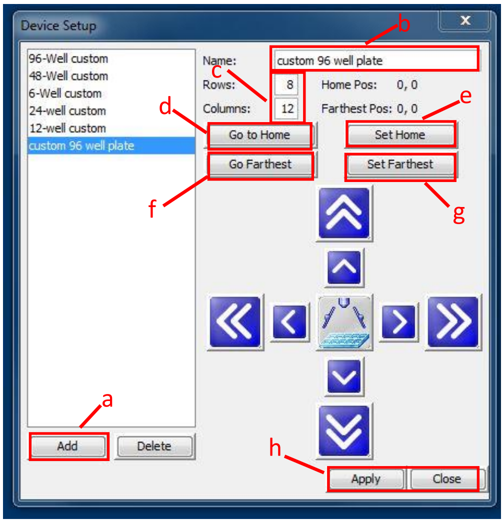
② Turn on the voltage and adjust the Far Left slider to minimal deflection to clear the hole in the splash shield (usually between16-18). Adjust other side streams sliders to 0.

③ Create a new *Sort Layout*.

④ Click the Access Stage button (⏏) in the Sort Layout to bring the ACDU stage to the front. Install a 96 well PCR plate sealed with an adherent film. Put A1 on the front left corner.

⑤ Click *Custom Devices* from Sort menu.





Name: custom 96 well plate
Rows: 8
Columns: 12
Home Pos: 0, 0
Farthest Pos: 0, 0

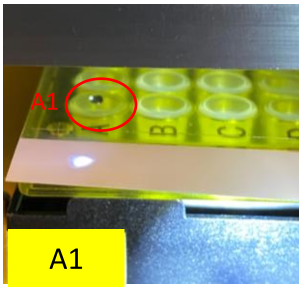
⑥ a. Add a new device.

b. Rename it.

c. Enter the number of rows and columns of custom device.

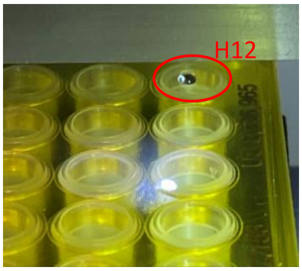
d. Click ***Go to Home***.

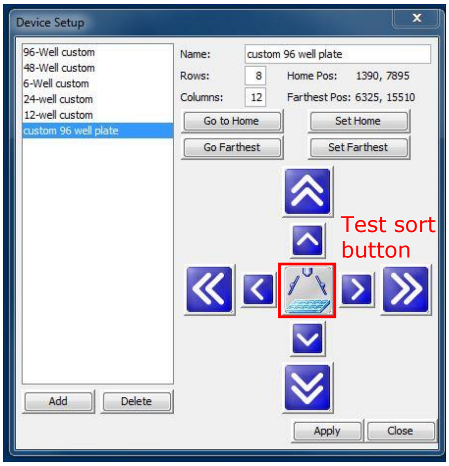
e. Move the ACDU stage by clicking the arrow buttons and the Test Sort button until drops deposit to the center of home location A1 well, then click ***Set Home***.



f. Click ***Go to Farthest***.

g. Move the ACDU stage by clicking the arrow buttons and the Test Sort button until drops deposit to the center of farthest location H12 well, then click ***Set Farthest***.





Name: custom 96 well plate
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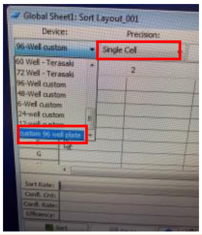
Columns: 12
Home Pos: 1390, 7895
Farthest Pos: 6325, 15510

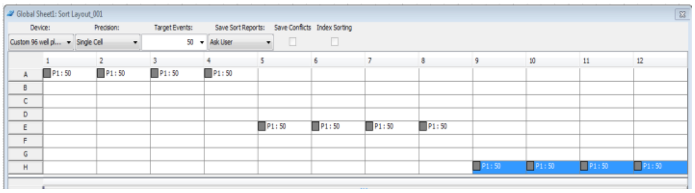
h. Click ***Apply*** and ***Close***. Now the custom device you created is listed in the Device menu in the Sort Layout window.



3. Test sort with beads

① Choose the custom device from the Device menu.

② Choose “Single cell” Precision mode.

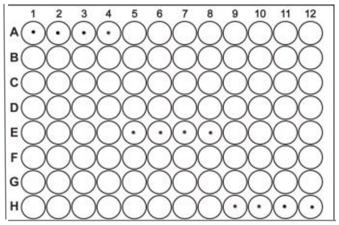


Device: Custom 96 well pl...
Precision: Single Cell
Target Events: 50
Save Sort Reports: Ask User
Save Conflicts ☐
Index Sorting ☐

	1	2	3	4	5	6	7	8	9	10	11	12
A	P1 : 50	P1 : 50	P1 : 50	P1 : 50								
B												
C												
D												
E					P1 : 50	P1 : 50	P1 : 50	P1 : 50				
F												
G												
H									P1 : 50	P1 : 50	P1 : 50	P1 : 50

③ Test sorting P1 50/well onto the cover for some wells with Accudrop beads or unstained control cells. The following wells (A1-4, E5-8, H9-12) are recommended for the calibration.

Note: Check ▸***Setting up for 96 well plate sorting SOP*** for detailed instructions.



④ Re-adjust the Home and Farthest position and repeat testing if needed.

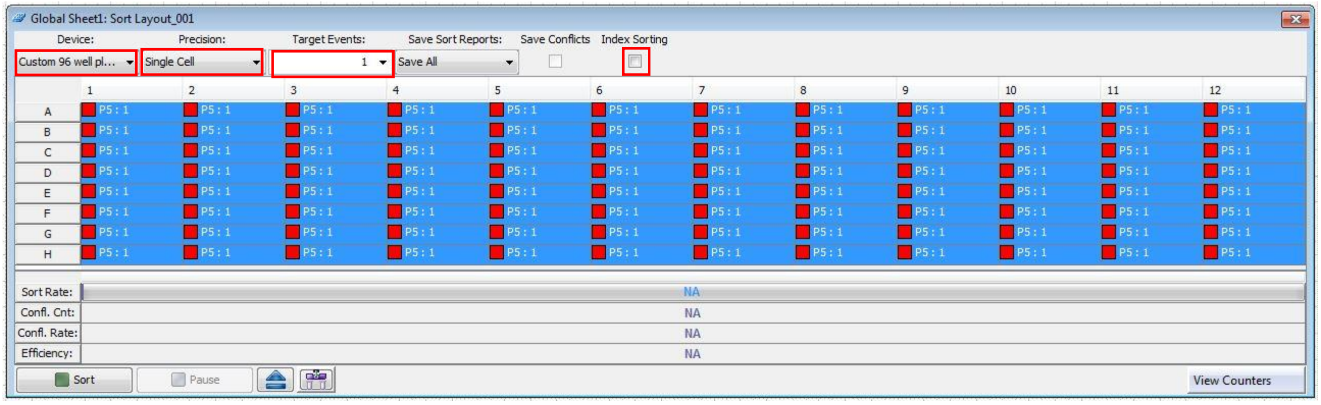
4. HRP method testing (optional)

HRP is a rapid and inexpensive colorimetric method to verify whether a well received a single droplet during plate sorting.

Note: See ▶***HRP verification for droplet deposition SOP***

5. Start sort experiment

1. Create a new *Sort Layout*.
2. Choose the custom device from the Device menu.
3. Choose “Single cell” Precision mode.
4. Select the wells and assign the gate and set the target number for each well.
5. Cleaning is required before acquiring and sorting sample. Usually clean with 1 minutes RINSE (detergent) at flow rate 11, followed by 1 minutes H2O at flow rate 11.
6. Load the collection plate on the ACDU stage. Make sure A1 on the front left corner. Open the plate lid or remove the adhesive sealing film.
7. Start acquiring the sample and start sorting. Index sort is available in Diva if needed.


Global Sheet1: Sort Layout_001

Device: Custom 96 well pl...

Precision: Single Cell

Target Events: 1

Save Sort Reports: Save All

Save Conflicts ☐

Index Sorting ☐

	1	2	3	4	5	6	7	8	9	10	11	12
A	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1
B	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1
C	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1
D	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1
E	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1
F	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1
G	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1
H	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1	P5 : 1

Sort Rate: NA

Confl. Cnt: NA

Confl. Rate: NA

Efficiency: NA